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BRIEF REPORT

Antibiotic Susceptibility Profile for the US *Neisseria meningitidis* Urethritis Clade

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The US *Neisseria meningitidis* urethritis clade (US_NmUC) harbors gonococcal deoxyribonucleic acid alleles and causes gonorrhea-like urogenital tract disease. A large convenience sample of US_NmUC isolates ($N = 122$) collected between January 2015 and December 2019 in Columbus, Ohio demonstrated uniform susceptibility to antibiotics recommended for gonorrhea treatment and meningococcal chemoprophylaxis.

Keywords. antibiotic resistance; antibiotic susceptibility; *Neisseria* spp; urethritis; US_NmUC.

In 2015, clusters of urethritis cases caused by a urethrotropic clade of *Neisseria meningitidis* (Nm) were identified at sexually transmitted disease (STD) clinics in the United States [1–5]. This pathogen, now known as the US *N meningitidis* urethritis clade (US_NmUC), has since been identified in the United Kingdom (UK) and Vietnam [6–8]. Several evolutionary processes likely contribute to the US_NmUC's urethrotropic nature, including the following: loss of capsule and lipooligosaccharide sialylation; acquisition of *Neisseria gonorrhoeae* (Ng) deoxyribonucleic acid (DNA), including a functional gonococcal denitrification pathway that facilitates microaerobic growth; enhanced resistance to antimicrobial peptides; and high surface expression of a unique factor H-binding protein, which enhances resistance to complement-mediated killing [2–5, 9, 10].

Antibiotics recommended for gonorrhea treatment are also recommended for Nm-associated urogenital infections [11, 12], and evidence suggests that they are effective for US_NmUC-related infections [2, 13]. However, there are reports of US_NmUC isolates with reduced susceptibility to penicillin, ciprofloxacin, and azithromycin [2, 6–8, 14, 15]. We have previously reported the absence of gonococcal antibiotic resistance alleles and known resistance determinants in US_NmUC isolates recovered in Columbus, Ohio [5]. In the present study, we extended these earlier results and correlate genomic findings with phenotypic antibiotic susceptibility results in our cohort of banked US_NmUC isolates. We examined antibiotics commonly used in STD clinic settings, including those recommended for gonorrhea treatment, and antibiotics recommended for meningococcal chemoprophylaxis [12, 16].

METHODS

Collection of US_NmUC Isolates

Between January 2015 and December 2019, we recovered 140 US_NmUC isolates from individuals seeking care at an urban STD clinic in Columbus, Ohio and were able to perform antibiotic susceptibility testing (AST) in 122 (87%). All isolates were confirmed to be US_NmUC by polymerase chain reaction and whole-genome sequencing (WGS), as previously described [2, 3]. The yearly number of isolates tested declined over time (2015 [$N = 69$], 2016 [$N = 36$], 2017 [$N = 12$], 2018 [$N = 3$], and 2019 [$N = 2$]), and most were recovered from the urethra of male patients ($N = 119$ of 122; 97.5%). One isolate was recovered from the rectum of a male patient ($N = 1$ of 122; 0.8%), 1 from the oropharynx of a male patient ($N = 1$ of 122; 0.8%), and 1 from the oropharynx of a female patient ($N = 1$ of 122; 0.8%).

Antibiotic Susceptibility Testing

We assessed the minimum inhibitory concentration (MIC) for penicillin, cefixime, ceftriaxone, ciprofloxacin, azithromycin, rifampin, tetracycline, and gentamicin using gradient diffusion E-test strips according to the manufacturer's instructions (bioMérieux, Inc.). In brief, 0.5 McFarland suspensions were inoculated onto Mueller-Hinton Agar supplemented with 5% sheep blood (Becton Dickinson and Co.) and incubated with E-test strips at 35–37°C in 5% CO_2 for 20–24 hours. Because

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higher azithromycin MICs have been reported when Nm is tested under 5% CO_2 compared with ambient air [17], we determined azithromycin MICs under both conditions. Finally, we confirmed penicillin MICs using broth microdilution (BMD) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. We based MIC interpretations (Table 1, footnote section) on CLSI guidelines for penicillin, ceftriaxone, ciprofloxacin, azithromycin, and rifampin [19] and on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for tetracycline [20]. Because no CLSI or EUCAST standards exist for Nm for cefixime and gentamicin, we used published interpretations for Ng [19, 21].

Genomic Analysis for Determinants of Decreased Antibiotic Susceptibility

We examined WGS data to identify alleles known to be associated with decreased antibiotic susceptibility using the genome comparator tool in PubMLST [22]. Antibiotic resistance mutations that are well described for the pathogenic *Neisseria* were examined by Clustal W alignment and included the following determinants: *penA*, *ponA*, *pilQ*, *mtrR/C/D/E*, and *porB* (β-lactam resistance), *gyrA*, *gyrB*, *parC*, and *parE* (fluoroquinolone resistance), 23S_rRNA, *rpsE*, and *mtrR/C/D/E* (macrolide resistance), *rpsJ* (tetracycline resistance), and *rpoB* (rifampin resistance). In addition to *mtrCDE*, we also checked genes encoding other efflux pumps (*farA/B*, *macA/B*, *marR*, and *norM*) and previously reported resistance mutations in *penA* (I312M, V316T, A501V, F504L, A510V, N512Y, I515V, H541N, G545S, P551L, and I566V), *gyrA* (T91F, T91I, and D95A), *mtrR* (G45D, A39T, and −35A deletion), *porB* (G120K and A121D), and *rpsJ* (V57M) [5, 6, 15, 23, 24]. Finally, we examined for the presence of bla_{TEM} (β-lactamase gene), which is associated with gonococcal penicillin resistance [25].

Ethical Approval

The Institutional Review Board at the Ohio State University approved this study.

RESULTS

All of the US_NmUC isolates were susceptible to ceftriaxone, ciprofloxacin, rifampin, and tetracycline (Table 1). Most isolates (75.4% by E-test and 98.4% by BMD) had intermediate penicillin susceptibility. Thirty isolates were penicillin-resistant by E-test, but all had intermediate susceptibility by BMD. Whereas 67.2% were azithromycin-susceptible under 5% CO_2, 100% were susceptible under ambient air. The MICs pertaining to cefixime (100%) and gentamicin (86.1%) for most isolates were in the susceptible range reported for Ng.

We identified no alleles that conferred phenotypic resistance to ceftriaxone, ciprofloxacin, azithromycin, rifampin, and tetracycline. All isolates had the *mtrR* allele 383, which is unique to the US_NmUC and does not carry mutations associated with elevated MtrCDE efflux pump activity [23]. All isolates had the *penA* allele 316, which carried F504L, A510V, N512Y, I515V, H541N, and I566V changes, but without other described mutations. The gonococcal bla_{TEM} was absent in all isolates.

Table 1. Minimum Inhibitory Concentration of US_NmUC Isolates (*N* = 122) to Select Antibiotics

Antibiotic Agent	MIC[a] Range	Interpretation[b]		
		S	I	R
Penicillin (BMD)	0.06–0.25	1.6%	98.4%	0%
Penicillin	0.064–0.5	0%	75.4%[c]	24.6%
Ceftriaxone	<0.002–0.004	100%	–	–
Ciprofloxacin	<0.002–0.012	100%	0%	0%
Rifampin	0.004–0.5	100%	0%	0%
Azithromycin, ambient air	<0.016–0.75	100%	–	–
Azithromycin, 5% CO_2	0.094–6	67.2%	–	–
Tetracycline	0.125–0.5	100%	–	0%
Cefixime	<0.016–<0.016	–	–	–
Gentamicin	1.5–6	–	–	–

Abbreviations: US_NmUC, US *Neisseria meningitidis* urethritis clade; BMD, broth microdilution; MIC, minimum inhibitory concentration; S, susceptible isolates; I, intermediate isolates; R, resistant isolates.

NOTE: Clinical and Laboratory Standards Institute (CLSI) MIC interpretative standards: penicillin, susceptible ≤0.06 μg/mL, intermediate = 0.12–0.25 μg/mL, and resistant ≥0.5 μg/mL; ceftriaxone, susceptible ≤0.12 μg/mL; ciprofloxacin, susceptible ≤0.03 μg/mL, intermediate = 0.06 μg/mL, and resistant ≥0.12 μg/mL; rifampin susceptible ≤0.5 μg/mL, intermediate = 1 μg/mL, and resistant ≥2 μg/mL; azithromycin susceptible ≤2 μg/mL. European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretative standard: tetracycline, susceptible ≤2 μg/mL and resistant >2 μg/mL. A dash mark (–) in the interpretation column indicates no CLSI or EUCAST standard exists for *N meningitidis* and for that specific category. For cefixime and gentamicin, published interpretation for the closely related pathogen *Neisseria gonorrhoeae* are as follows: cefixime, susceptible ≤0.25 μg/mL; gentamicin, susceptible ≤4 μg/mL, intermediate = 8–16 μg/mL, and resistant ≥32 μg/mL.

[a]MIC μg/mL; unless otherwise noted, the MIC values were determined using the E-test.

[b]S, I, and R given as the percentages of all (*N* = 122) isolates tested.

[c]Three isolates with penicillin MIC = 0.064 μg/mL by E-test were categorized as having intermediate susceptibility; 2 were subsequently determined to be susceptible (MIC = 0.06 μg/mL) and 1 intermediate (MIC = 0.125 μg/mL) by BMD.

DISCUSSION

Contrary to observations in Ng, widespread resistance to clinically relevant antibiotics in Nm remains rare [23, 26–28]. However, the US_NmUC has acquired gonococcal DNA over time [3, 5], including alleles associated with decreased antibiotic susceptibility [5–8]. Whereas antibiotics recommended for gonorrhea treatment appear to remain effective for treating US_NmUC-related urogenital infections, phenotypic antibiotic susceptibility analyses have been reported in a limited number of isolates, with some reporting decreased penicillin, ciprofloxacin, and azithromycin susceptibilities [2, 4, 6–9, 14, 15]. A ciprofloxacin-resistant US_NmUC rectal isolate (MIC = 0.38 mg/L) in the UK had acquired a partial gonococcal *gyrA* allele 9 (with T91F and D95A) [6]. Eight of 19 US_NmUC isolates from Vietnam contain the same T91F and D95A mutations in the *gyrA* allele 381. An additional Vietnam isolate

had a T91I mutation in the *gyrA* allele 382. The MICs of these 9 isolates ranged from 0.19 to 3 mg/L [7, 8]. Retchless et al [5] reported that a urethral isolate from New York had acquired a gonococcal-like *mtrR* sequence (allele 39) associated with elevated azithromycin MICs, whereas Sukhum et al [15] reported decreased azithromycin susceptibility for 7 of 8 urogenital isolates with testing performed under 5% CO_2.

The aforementioned findings indicate that antibiotic resistance determinant acquisition is a concern in the US_NmUC. However, among US_NmUC isolates collected from 2015 to 2019 in Columbus, Ohio, we correlated the absence of genotypic resistance determinants with the phenotypic susceptibility to antibiotics recommended for gonorrhea treatment (ie, ceftriaxone) [12] and meningococcal chemoprophylaxis (ie, ceftriaxone, ciprofloxacin, and rifampin) [16]. Many US_NmUC isolates had decreased azithromycin susceptibility when tested under 5% CO_2 conditions, but not under ambient air. Although the clinical significance of these in vitro findings are not known, they agree with previous reports of elevated azithromycin MICs when testing occurs under CO_2-enriched conditions [17].

Most US_NmUC isolates had intermediate penicillin susceptibility, but they were susceptible to ceftriaxone and had very low cefixime MICs. The chromosomally mediated penicillin resistance in Ng is attributed to 5 mutated resistance determinants (*penA*, *ponA*, *porB*, *mtr*, and *pilQ*), which can be transferred to a susceptible strain by homologous recombination [29]. *Neisseria meningitidis* and Ng with reduced susceptibility to penicillin commonly harbor alterations in the *penA* gene encoding the penicillin binding protein 2 (PBP2). The mosaic-like structure of the *penA* gene, with ~60 amino acid alterations, has evolved by homologous recombination with *penA* genes of commensal *Neisseria* species [30] and is associated with reduced cefixime susceptibility. Three mutations (G545S, I312M, and V316T), all absent in the tested US_NmUC isolates, were proposed to be responsible for reduced cefixime susceptibility [31], but only in the presence of other amino acid changes that have little apparent effect alone [32]. An L421P substitution in *ponA* (PBP1), together with overexpression of the MtrCDE efflux pump and mutations in porin (PorB) and the type IV pilin channel (PilQ), were involved in high-level penicillin resistance [33]. These mutations were absent from our isolate collection [5]. Finally, we did not perform phenotypic β-lactamase testing, but the gonococcal bla_{TEM} was absent in all isolates [25], and the observed intermediate penicillin susceptibility does not support the presence of other β-lactamases, such as the one encoded by $bla_{ROB\text{-}1}$, which has been reported in invasive Nm serogroup Y isolates and confers high-level penicillin resistance (>2 mg/L) [26, 34]. Overall, our findings support the clinical observation that patients diagnosed with US_NmUC urethritis did not experience treatment failure after receiving ceftriaxone-based regimens [2, 13].

We note several important study limitations. All isolates that underwent AST were collected at one STD clinic; therefore, the findings may not represent the susceptibility profile of US_NmUC isolates circulating elsewhere. The US_NmUC evolution has been characterized by acquisition of gonococcal DNA, including genes associated with antibiotic resistance. Given the cohabitation of US_NmUC and Ng, ongoing surveillance is critical to determine whether US_NmUC isolates continue to acquire antibiotic resistance genes.

CONCLUSIONS

The US_NmUC isolates from Columbus, Ohio were susceptible to antibiotics used for gonorrhea treatment and for meningococcal chemoprophylaxis. However, given that this emerging urethrotropic Nm clade shares an ecologic niche with—and has acquired genes from—Ng, ongoing surveillance is warranted to monitor for the development and spread of antibiotic resistance.

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Author contributions. JAB, Y-LT, DSS, JLE, and ANT formulated and designed the research project; AC and BS collected data; Y-LT and SWS performed the genomic analyses and antibiotic susceptibility testing, respectively. JAB, Y-LT, KMB, SWS, and ANT analyzed and interpreted the data and drafted the manuscript; all authors critically reviewed and approved the manuscript.

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